

REMARKS

Claims 1, 3-5, 8-13, and 25-30 will be pending in the application upon entry of the proposed amendments. Claims 1 and 25 have been amended to specify the activity of the β -galactosidase associated with a bacterium at 4°C and 55°C. Support for the amendment is found throughout the specification, e.g., at page 24, line 27-page 25, line 6, and in Fig. 12. The specification has been amended to add the filing date of the provisional application from which the present application claims priority and to correct a typographical error (support at 11, line 30).

No new matter is introduced by the amendment.

Applicant thanks the Examiner for the helpful discussion with applicant's representatives on July 10, 2003.

In view of the amendments and arguments presented below, applicant respectfully requests entry of the amendment and reconsideration of the claims, which applicant believes are now in condition for allowance.

The Invention

The pending claims are drawn to a method of preparing a lactase microcarrier (e.g., independent claim 1) and a permeabilized bacterium (e.g., independent claim 25) and have been amended to incorporate the surprising results disclosed in the specification, specifically, rapid lactose hydrolysis at 55°C and 4°C. The claimed method includes transforming a food grade lactic acid bacterium with a construct that includes a lantibiotic promoter that is operatively linked to a sequence encoding a β -galactosidase (BG), culturing the bacterium under conditions such that the bacterium contains sufficient BG to exhibit at least 4,000 MU, and permeabilizing the bacterium. The permeabilized bacterium has at least one of the following characteristics related to increased lactose hydrolysis: when present at an OD₆₀₀ of about 1.55 it can (1) hydrolyze about 100% of the lactose in skim milk at a temperature of 55°C within two to three hours, or (2) hydrolyze at least about 50% of the lactose in skim milk at a temperature of 4°C within two to three hours, or (3) both (1) and (2). The permeabilized bacterium of claim 25 has characteristics like those of the microcarrier produced by the claimed method.

The Examiner indicated in his interview with applicant's representative that inclusion of these results in the claims may constitute allowable matter.

35 U.S.C. § 103 (a)

Claims 1, 3-5, 8-13, and 25-30 have been rejected for alleged obviousness over Somkuti (a) et al. (Enzyme Microb. Technol., 1994, 16:573-576), Somkuti (b) et al. (Curr. Microbiol., 1998, 36:202-206), Herman et al. (In: Streptococcal Genetics, Ferretti and Curtiss (Eds.), pp. 225-228, 1987), VanBelkum et al. (J. Bacteriol., 1991, 173: 7934-7941), Lee et al. (Biotechnol. Bioeng., 1996, 52:572-578), and Chang et al. (U.S. Patent No. 5,766,907, 6/16/98). Applicant respectfully traverses the rejection in view of the following reasons.

First, applicant maintains that the Office Action has failed to establish a *prima facie* case of obviousness. Applicant will review his position on this point in further detail below. Second, even assuming that the Office Action has established a proper obviousness rejection, applicant's claimed invention provides unexpected results that clearly rebut the rejection.

Unexpected Results and Claim Scope

In the previous Response (at page 11) applicant stated

The present invention provides not only the unexpected result of greater efficiency at low temperature, but also the distinct advantage of incubation at low temperatures, which can streamline the production of lactose-free milk by reducing incubation time and allowing production in a single operating day. In addition, it is known in the art related to dairy processing that psychotrophs, bacteria that grow at cold temperature, can adversely affect the flavor of milk. Conventional BG treatments can be used at low temperature, however, they require long incubation times (e.g., 16-24 hours), which reduce production efficiency and permit psychotroph growth. The invention provides an unexpectedly efficient method of hydrolyzing lactose such that the hydrolysis can be carried out at low temperature, but still in a relatively short time. This can reduce psychotroph growth, thereby reducing the chance of such organisms causing an off-flavor to BG-treated milk. The invention therefore fulfills a need in the art and provides unexpected advantages.

In addition, applicant showed a surprising result of efficient lactose hydrolysis in skim milk at 55°C (see specification, e.g., at Example 9 and Fig. 12). These features of efficient lactose hydrolysis at either high or low temperatures, or both are now incorporated into the claims. The Examiner indicated in the interview with applicant's representatives that he would consider whether an amendment of this type would constitute allowable matter. In view of the fact that the Examiner appears to agree that applicant has provided at least some measure of "unexpected results," and to expedite prosecution, applicant has incorporated language into the independent claims to reflect these results. Applicant believes that the scope of the claims is clearly commensurate with the unexpected results. Thus, regardless of the Examiner's view of the discussion above related to cited references, applicant believes that in view of the amendment, all of the claims are allowable.

With respect to the Office Action's assertion that "applicant has not shown that the high level of BG production can be achieved using **any promoter in any lactic acid bacteria** or all the species of the lactic acid bacteria claimed in claim 3" (page 6, second full paragraph, emphasis in original), applicant has amended the claims such that the promoter is a lantibiotic promoter (e.g., the nisA promoter). Applicant also believes that he has shown that bacteria having the claimed characteristics can be made and that one in the art would be able to determine whether a bacterium was within the scope of the claim.

Cited References

According to the Office Action

Somkuti (a), (b), and VanBelkum et al. references were used to show that the technique of permeabilization of lactic acid bacteria such that the BG enzyme in the cells would be easily accessible for the hydrolysis of the lactose in the milk product was very well known in the art to solve the problem of lactose intolerance due to consumption of dairy products (Office Action at page 3).

Claim 1 now requires a bacterium that contains a promoter from a lantibiotic gene. Furthermore, as per the amendment to claim 1, the claimed microcarrier must provide at least one of unexpected results disclosed in the application that feature improved lactose hydrolysis:

at a concentration of about 1.55 at OD₆₀₀, the bacteria can hydrolyze about 100% of lactose at 55°C in 2-3 hours, can hydrolyze at least about 50% of lactose at 4°C in 2-3 hours, or both.

Amended claim 25 also requires that the claimed bacterium have these features.

None of Somkuti (a), (b), or VanBelkum et al. discloses the use of a BG sequence under the control of a lantibiotic promoter in any cell, much less in a lactic acid bacterium, nor do these references disclose cells that have the features of improved lactose hydrolysis at 4°C or 55°C that have been added by the present amendment. Since these references do not disclose all of the elements of the claims, the Office Action looks to other art to supply the deficiencies.

However, neither Herman nor Lee et al. disclose making a lactic acid bacterium making BG under the control of a lantibiotic promoter. Furthermore, neither of these references discloses or suggests that one could have made a lactic acid bacterium or microcarrier that has the features of improved lactose hydrolysis at 4°C or 55°C, as required by the present claims.

The Office Action also states, "Applicants argue as if claim 1 was limited to promoters that work only in lactic acid bacteria" (Office Action at page 4, first paragraph). Applicant does not dispute this. Claim 1 requires expression of β -galactosidase operatively linked to a lantibiotic promoter sequence, and the β -galactosidase must be expressed in an amount sufficient to exhibit a BG activity of at least 4,000 Miller Units. Thus, the claim is *de facto* limited to promoters that work only in lactic acid bacteria.

Chang et al. is cited against claim 29,

Chang et al. reference was used to show the advances in packaging or immobilization of microbial cells, which is claimed in claim 29. Even though it was explain in the rejection, it appears that the applicant has missed. (Office Action at page 4, first full paragraph)

Chang et al. does not supply the deficiencies of Somkuti (a), (b), VanBelkum et al., Lee et al., or Herman et al., and thus cannot make obvious claim 29, which depends from claim 1. Accordingly, Chang et al., combined with the other cited references, cannot make obvious claim 29.

As the Examiner knows, to establish a *prima facie* case of obviousness, three criteria must be met. First, there must be some suggestion or motivation, either in the references

themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

Applicant believes that the references do not provide all of the elements of the claims, nor do the references provide any suggestion or motivation to combine the references. In view of these deficiencies, it is unclear how one could have a reasonable expectation of success.

In the final Office Action it was stated that applicant has argued "as if each and every one of the reference [sic] must have disclosed or taught the invention in order to render the invention obviousness (Office Action at paragraph bridging pp. 2-3). However, that is not the case. Applicant understands that all of the elements need not be disclosed in a single reference, but it is required that the suggestion or motivation to combine the elements to make the claimed invention be provided by the art. However, the Office Action fails to point to such specific motivation (rather than a general, vague suggestion) in the cited references.

Applicant maintains that the cited references, even when combined, do not provide all of the elements of the claimed invention, nor do any of the cited references provide a motivation or suggestion to combine elements to make the claimed invention. As the Examiner knows "[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination." (In re Mills, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

Use of S. thermophilus in Industry

Applicant stated in the previous Response (dated July 26, 2002), that *S. thermophilus* BG is not the most favored in the industry and provided evidence to support this statement (page 7, first full paragraph) noting that a reference cited by the Office Action, Somkuti (b) states (at page 202, column 2, first full paragraph) "[a]t present, low-lactose milk is produced primarily with β -gal isolated from yeast (*Kluyveromyces* sp.)..." In the present Office Action, the Examiner states that he "made the conclusion that the *S. thermophilus* is the most favored in the industry based on his general survey of the literature and also based on the reference of Lee et al. who

have used it for high level production of BG” (Office Action at page 5, first paragraph). There is no explanation as to why the Office Action dismisses, without explanation, Somkuti et al.’s statement that *Kluyveromyces sp.* is the primary source of BG. The Office Action fails to provide any information as to how the survey was conducted as well as any specific reference to the literature surveyed. Applicant therefore requests that this aspect of the rejection be withdrawn. If the rejection is maintained, applicant requests an explanation as to why the statement of Somkuti (b) was dismissed and copies of the references referred to by the Office Action as discovered in the survey.

The Office Action also states “the conclusion that the *S. thermophilus* is most favored in the industry is ...based on the reference of Lee et al. who have used it for high level production of BG (Office Action at page 5, lines 2-3). Applicant respectfully submits that Lee et al. used *E. coli*. The Examiner wonders why the yeast gene was not chosen for high level production of BG by Lee et al. (Office Action at page 5, first paragraph). Applicant points out that Lee et al. is not concerned with the commercial production of BG for milk. Instead, as discussed above, Lee et al. is concerned with improving the production of proteins specifically using *E. coli* “as the proteins can be produced by *E. coli* rapidly and inexpensively” (Lee et al. at page 572). Thus, Lee et al. is concerned with inducible promoters to be used for commercial applications in *E. coli*. As discussed above, Lee et al. does not supply the deficiencies of the other cited references and since Lee et al. is concerned with a problem related to production of proteins in *E. coli*, applicant submits that there is no reason to believe that one would look to Lee et al. to make the present invention. Certainly Lee et al. provides no motivation or suggestion to combine elements provided by the cited references to make the claimed invention.

Claims 6-9

Claims 6-9 are rejected for alleged obviousness in view of Somkuti (a), (b), Herman et al., VanBelkum et al., and Lee et al. as applied to claims 1 and 3-5, 10-13, and 25-30 above, and further in view of Kuipers et al. Claims 6 and 7 have been cancelled as their subject matter is incorporated by the present amendment into claim 1.

Claims 8 and 9, which depend from claim 1 are pending and are drawn to the use of a promoter for expression of BG enzyme in the recombinant lactic acid bacteria, such that the promoter is from a gene encoding a lantibiotic such as a nisin gene promoter, nisA.

First, applicant maintains "[d]ependent claims are nonobvious under section 103 if the independent claims from which they depend are nonobvious." *In re Fine*, 5 U.S.P.Q.2d 1596, 1600 (Fed. Cir. 1988) (emphasis added). As argued above, applicant maintains that claim 1, from which claims 8 and 9 depend, is not obvious. Therefore, claims 8 and 9 cannot be obvious.

Furthermore, the addition of Kuipers cannot make up for the deficiencies of the other cited references. Kuipers et al. discloses a method for gene expression in lactic acid bacteria by providing a DNA fragment that is under the control of a promoter for a nisA promoter of the nisin gene (an antimicrobial peptide) from *L. lactis*. The Office Action describes a number of "advantages" of using the nisA promoter. However, the reference does not disclose or suggest that one could have made a bacterium or microcarrier having the features of improved lactose hydrolysis at 4°C or 55°C.

The Office Action also discusses Lee et al. with respect to claims 6-9. As discussed above, this reference does not describe permeabilization of cells, high BG activity at low temperature, or anything related to a use that would involve food-grade bacteria, i.e., a lactic acid bacterium. Importantly, Lee et al. does not provide any suggestion or motivation to combine elements to make the claimed invention.

The Office Action states

Examiner has listed a number of motivational reasons among which the following would be one of the more compelling reasons for one skilled in the art of making dairy products for human consumption, i.e., the reference teaches that advantageously, while the induction of the **preferred gene** can be obtained as a positive regulation, only very small amounts i.e., less than 1 microgram/liter of the inducing factor will be required (see column 6) which does not greatly alter the food/milk composition and the antimicrobial activity of nisin is also another added advantage which kills the growth of any opportunistic contaminant in milk." (Office Action at paragraph bridging pages. 8-9, emphasis in original).

Applicant's point is that the "motivational reasons" are provided by the Office Action and not by the art. The motivation to combine the references must come from the art for a proper rejection. Accordingly, in addition to the assertion that the combined cited references do not provide all of the elements of the claimed invention, there is no art-initiated motivation or suggestion to combine the references and the claims cannot be made obvious.

In view of the amendments to the claims and the arguments presented above, applicant respectfully requests withdrawal of the rejection under 35 U.S.C. § 103.

CONCLUSION

Applicant submits that all claims are in condition for allowance, which action is respectfully requested. This Reply is being filed with a Request for Continued Examination.

Enclosed is a \$740.00 check for the Petition for Extension of Time fee. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing attorney docket number 11072-002001.

Respectfully submitted,

Date: November 5, 2003



Lisa N. Geller, Ph.D., J.D.
Reg. No. 51,726

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906